RESULTS

1. ANTI-INFLAMMATORY ACTIVITY

1.1 Effects of GH5763 and phenylbutazone on EPP-induced ear edema in rats

The inhibitory effect produced by the topical administration of GH5763 on EPP-induced rat ear edema was assessed. As shown in Table 1, in the control group (received acetone), edema thickness was 36.67 ± 3.33, 93.33 ± 4.22, 163.33 ± 9.54 and 156.67 ± 6.15 μm at 15, 30, 60 and 120 min after EPP application, respectively. Phenylbutazone, a nonsteroidal anti-inflammatory drug, at a dose of 1 mg/ear produced significant inhibitory activity on edema formation at all determination times. It produced marked antiedema activity of 73%, 68%, 63% and 43% at 15, 30, 60 and 120 min, respectively. At a dose of 1 mg/ear, GH5763 also possessed profound inhibitory effect on EPP-induced rat ear edema at all assessment times. This dose showed significant inhibitory effect on edema formation of 82%, 93%, 86% and 72% at 15, 30, 60 and 120 min, respectively, after topical application of EPP.

1.2 Effects of GH5763 and aspirin on carrageenin-induced hind paw edema in rats

The inhibitory activity on carrageenin-induced rat hind paw edema caused by an oral administration of GH5763 and aspirin at various time after carrageenin injection is shown in Table 2.

Table 1. Effects of GH5763 and phenylbutazone on EPP-induced ear edema in rats

Group	Dose (mg/ear)	Time after topical application of EPP							
		15 min		30 min		1h		2 h	
		ED (μm)	EDI (%)	ED (μm)	EDI (%)	ED (μm)	EDI (%)	ED (μm)	EDI (%)
Control	-	36.67 ± 3.33	-	93.33 ± 4.22	-	163.33 ± 9.54	-	156.67 ± 6.15	-
Phenylbutazone	1	10.00 ± 4.47***	73	30.00 ± 4.47***	68	60.00 ± 10.33***	63	90.00 ± 4.47***	43
GH5763	1	6.67 ± 4.22***	82	6.67 ± 4.22***	93	23.33 ± 12.02***	86	43.33 ± 9.54***	72

Test drugs were applied topically to both inner and outer surfaces of the ear. Control = received vehicle (acetone) only.

Values are expressed as mean ± S.E.M. ($N = 6$). Significantly different from control: ***$P < 0.001$.

ED = edema thickness , % ED = percent edema inhibition

Table 2. Effects of GH5763 and aspirin on carrageenin-induced paw edema in rats

Group	Dose (mg/kg)	Time after carrageenin injection					
		1 h		3 h		5 h	
		EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
Control	-	0.47 ± 0.08	-	0.81 ± 0.04	-	0.72 ± 0.06	-
Aspirin	300	0.16 ± 0.01***	66	0.25 ± 0.03***	69	0.24 ± 0.04***	67
GH5763	10	0.28 ± 0.03**	40	0.53 ± 0.02***	36	0.44 ± 0.04***	39
	20	0.23 ± 0.03**	51	0.42 ± 0.04***	48	0.38 ± 0.03***	47
	40	0.19 ± 0.04***	60	0.34 ± 0.04***	58	0.32 ± 0.03***	56

Test drugs were orally administered 1 h before carrageenin injection. Control = received 5% Tween 80 only.

Values are expressed as mean ± S.E.M. ($N = 6$). Significantly different from control: $^{**}P < 0.01$, $^{***}P < 0.001$.

EV = edema volume (ml) , % EI = percent edema inhibition

Aspirin, a cyclooxygenase inhibitor, at the dose of 300 mg/kg exhibited significant edema inhibitory activity. GH5763 at doses of 10, 20 and 40 mg/kg possessed profound inhibitory effect on carrageenin-indeced paw edema at all assessment times. Three hours after carrageenin injection, GH5763 at doses of 10, 20 and 40 mg/kg elicited inhibitory effect on the paw edema formation of 36%, 48% and 58%, respectively. The percent edema inhibition produced by the dose of 300 mg/kg of aspirin on the paw edema formation was found to be at the 3rd h after carrageenin injection.

1.3 Effects of GH5763, aspirin and phenidone on AA-induced hind paw edema in rats

Results obtained from the hind paw edema induced by AA are demonstrated in Table 3. AA evoked edematous response when it was injected into the plantar side of the right hind paw. The average edema volume in the control group amounted to 0.51 $\pm$ 0.03 ml, at 1 h after AA injection. At doses of 10, 20 and 40 mg/kg, GH5763 did not elicite significant inhibitory effect on AA-induced hind paw edema. Similarly, aspirin at the dose of 300 mg/kg did not show any inhibitory effect on AA-induced edema. By contrast, phenidone, a dual inhibitor of AA metabolism, exhibited marked inhibitory activity on the edema formation of 41% when assessment was done 1 h after injection. The results showed that GH5763 had no inhibitory effect on the edema formation induced by AA.

Table 3. Effects of GH5763, aspirin and phenidone on arachidonic acid (AA)-induced paw edema in rats.

Group	Dose (mg/kg)	EV (ml)	EI (%)
Control	-	0.51 ± 0.03	-
Aspirin	300	0.47 ± 0.04	8
Phenidone	40	0.30 ± 0.03	41***
GH5763	10	0.50 ± 0.03	2
	20	0.45 ± 0.03	12
	40	0.43 ± 0.03	16

Test drugs were orally administered 2 h before AA injection. The paw volume was measure prior to and 1 h after AA injection.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: *** $p < 0.001$.

Control = received 5% Tween 80 only.

EV = Edema Volume; EI = Edema inhibition

1.4 Effect of GH5763, aspirin and prednisolone on the cotton pellet-induced granuloma formation

1.4.1 Granuloma formation and transudation

The inhibitory effects of GH5763 and the two reference drugs, i.e. aspirin and prednisolone, on the cotton pellet-induced granuloma formation in rats were examined on the eight day after the daily oral administration of test drugs for 7 days. The inhibitory activities of GH5763 and the reference drugs on the granuloma formation induced by cotton pellet implantation are shown in Table 4. It was found that aspirin and GH5763, at the dose of 300 mg/kg and 20 mg/kg, respectively, exhibited slight inhibitory effect on the granuloma formation of 20% and 18%, respectively. Prednisolone, on the contrary, elecited marked antigranuloma formation of 44%.

The transudative weight of the control group was found to be 214.51 $\pm$ 22.67 mg. Aspirin, prednisolone and GH5763, significantly reduced the weight of the transudate to 151.12 $\pm$ 19.63, 116.32 $\pm$ 10.03 and 163.93 $\pm$ 6.16 mg, respectively. It indicates that all test drugs inhibited the formation of the transudate.

1.4.2 Body weight gain and thymus weight

Results demonstrated in Table 5 show the body weight gain during the first and the last day of the experimental period and the dry weight of thymuses of the rats implanted with cotton pellets. In the control group the body weight gain in one week was 39.17 $\pm$ 1.14 g. Both aspirin (300 mg/kg) and GH5763 (20 mg/kg) did not affect the body weight gain of animals. The gain of the weight in aspirin and GH5763-treated groups were 36.33 $\pm$ 1.28 and 36.00 $\pm$ 1.81 g, respectively, which were not significantly different from that of control group. In contrast, prednisolone, at the dose of 5 mg/kg significantly reduced the gain of the body weight to 21.00 $\pm$ 4.03 g.

Table 4. Effects of GH5763, aspirin and prednisolone on granuloma formation and transudation of cotton pellet-induced granuloma in rats

Group	Dose (mg/kg)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)	GI (%)
Control	-	273.71 ± 26.09	59.20 ± 3.68	214.51 ± 22.67	1.96 ± 0.18	-
Aspirin	300	202.45 ± 20.35*	51.33 ± 1.87*	151.12 ± 19.63*	1.56 ± 0.09*	20
Prednisolone	5	158.32 ± 10.69***	41.99 ± 2.15***	116.32 ± 10.03***	1.10 ± 0.11***	44
GH5763	20	215.96 ± 6.94*	52.02 ± 1.13*	163.93 ± 6.16*	1.60 ± 0.05*	18

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.05$, *** $P < 0.001$

GI = granuloma inhibition. Control = received 5% Tween 80 only.

Table 5. Effects of GH5763, aspirin and prednisolone on body weight and thymus weight of cotton pellet-induced granuloma in rats

Group	Dose (mg/kg)	Body weight (g)			Dry thymus weight (mg/100 g)
		Initial	Final	Gain	
Control	-	180.00 ± 2.88	219.17 ± 2.76	39.17 ± 1.14	48.51 ± 2.94
Aspirin	300	181.67 ± 1.67	218.00 ± 1.88	36.33 ± 1.28	45.58 ± 1.96
Prednisolone	5	181.33 ± 2.89	202.33 ± 5.15**	21.00 ± 3.70***	21.39 ± 2.67***
GH5763	20	181.67 ± 1.30	217.67 ± 2.14	36.00 ± 1.81	45.37 ± 2.49

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: ** $P < 0.01$, *** $P < 0.001$.

Control = received 5% Tween 80 only.

Dry thymus weight of rats in control group was 48.51 ± 2.94 mg/100 g body weight. Both aspirin and GH5763 did not show any suppressive effect on the thymus weight (45.58 ± 1.96 and 45.37 ± 2.49 mg/100 g body weight, respectively) of the rats when compared with that of the control group, whereas prednisolone significantly reduced the thymus weight of the animals to 21.39 ± 2.67 mg/100 g body weight.

1.4.3 Alkaline phosphatase activity

The effects of test drugs on alkaline phosphatase activity in rats implanted with cotton pellets are shown in Table 6. Significant elevated alkaline phosphatase level in the serum of rats in control group was observed (38.97×10^{-4} U of enz./mg of serum protein) when compared with that of normal or non-implanted rats (26.95×10^{-4} U of enz./mg of serum protein). The increase in serum alkaline phosphatase caused by cotton pellet implantation was reduced to normal level by GH5763 at the dose of 20 mg/kg (26.03×10^{-4} U of enz./mg of serum protein) as well as aspirin at the dose of 300 mg/kg (26.02×10^{-4} U of enz./mg of serum protein) and prednisolone at the dose of 5 mg/kg (27.40×10^{-4} U of enz./mg of serum protein).

2. ULCEROGENIC ACTIVITY

2.1 Effects of GH5763 and aspirin on gastric mucosa in rats

Results demonstrated in Table 7 show the effect of GH5763 in comparison with aspirin and prednisolone on the gastric mucosa of rats on the eight day after the daily oral administration of test drugs for 7 days. It was found that aspirin at the dose of 300 mg/kg/day and prednisolone at the dose of 5 mg/kg/day caused significant gastric

Table 6. Effects of GH5763, aspirin and prednisolone on alkaline phosphatase activity in serum of cotton pellet-induced granuloma in rats

Group	Dose (mg/kg)	Alkaline phosphatase (units/l)	Total protein (g/dl)	Serum alkaline phoshatase activity (U of enz./mg of serum protein x 10^{-4})
Normal	-	117.50 ± 1.91	4.37 ± 0.08	26.95 ± 0.63
Control	-	182.17 ± 12.39	4.70 ± 0.19	38.97 ± 2.73[a]
Aspirin	300	116.17 ± 4.60	4.48 ± 0.12	26.02 ± 1.30[b]
Prednisolone	5	138.50 ± 7.02	5.05 ± 0.21	27.40 ± 0.45[b]
GH5763	20	117.17 ± 4.22	4.53 ± 0.13	26.03 ± 1.47[b]

Values are expressed as mean ± S.E.M. ($N = 6$).

a = significant from normal: $P < 0.001$.

b = significant from control: $P < 0.001$.

Normal = non-implanted group

Control = implanted group, received 5% Tween 80 only.

Table 7. Effects of GH5763, aspirin and prednisolone on gastric mucosa

Group	Dose (mg/kg)	Ulcer index
Control	-	0
Aspirin	300	1**
Prednisolone	5	2**
GH5763	20	0

Values are expressed as median ($N = 6$).

Significantly different from control: ** $p < 0.01$

Control = received 5% Tween 80 only.

ulceration with ulcer index of 1 and 2, respectively. On the contrary, GH5763 did not affect the gastric mucosa of the rats compared with those in the control group.

3. ANALGESIC ACTIVITY

3.1 Effects of GH5763 and aspirin on acetic acid-induced writhing response in mice

Writhes were induced in mice by an intraperitoneal injection of 0.75% acetic acid aqueous solution. Aspirin and GH5763 were administered orally 1 h before the acetic acid injection.

The results of the acetic acid-evoked writhing response in mice are given in Table 8. In the control group, the intraperitoneal injection of acetic acid produced 57.50 $\pm$ 2.22 writhes during the period of 15 min, beginning of 5 min after acetic acid injection. Aspirin at a dose of 300 mg/kg exerted significant inhibition of the number of writhes with the percentage of inhibition of 79. At doses of 10, 20 and 40 mg/kg, GH5763 similarly exerted significant inhibitory activity on writhing response with the percentages of 33, 43 and 56, respectively.

3.2 Effects of GH5763 and aspirin on the formalin test in mice

The inhibitory effect of GH5763 on algesia induced by formalin injection at the hind paw of mouse was investigated both in early phase and late phase, using licking of the paw as a criterion for algesia.

Table 8. Effects of GH5763 and aspirin on acetic acid induced writhing response in mice

Group	Dose (mg/kg)	No. of writhes	Inhibition of Writhing Response (%)
Control	-	57.50 ± 2.22	-
Aspirin	300	11.83 ± 0.94***	79
GH5763	10	38.67 ± 0.99***	33
	20	32.67 ± 1.38***	43
	40	25.50 ± 0.96***	56

Test drugs were orally administered 1 h before acetic acid injection.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: *** $P < 0.001$

Control = received 5% Tween 80 only.

3.2.1 Early phase

The results of licking response in the early phase of the formalin test are shown in Table 9. The licking time of the control group was 91.56 sec. GH5763 showed a significant inhibitory effect on the licking response causing a reduction of licking time. The doses of 10, 20 and 40 mg/kg of GH5763 decreased the licking time to 61.42, 54.44 and 41.66 sec, with the percent inhibition of 33, 40 and 54, respectively. Aspirin, at a dose of 300 mg/kg slightly reduced the licking time to 69.71 sec with percent inhibition of 24.

3.2.2 Late phase

Inhibition of licking response of the test drugs in the late phase of the formalin test is shown in Table 10. The licking time of the control group was 97.76 sec whereas thoses of GH5763, at doses of 10, 20 and 40 mg/kg were 68.32, 44.85 and 26.68 sec, with percent inhibition of 30, 54 and 73, respectively. At the dose of 300 mg/kg, aspirin showed 57% reduction of licking time.

4. ANTIPYRETIC ACTIVITY

4.1. Yeast-induced hyperthermia in rats

As shown in Table 11 for rats in the group which received aspirin at the dose of 300 mg/kg significant reduction of the rectal temperature was observed. Aspirin could reduce the rectal temperature of the rats from $39.37 \pm 0.31\ ^{\circ}C$ to 38.47 ± 0.28, 38.07 ± 0.14, 37.73 ± 0.14 and $37.47 \pm 0.27\ ^{\circ}C$ when measurement was made 30, 60, 90 and 120 min, respectively, after drug administration. GH5763, at a dose of 10 mg/kg, elicited

pronounced effects in reduction of rectal temperature after yeast-induced pyrexia from 39.03 ± 0.07 °C to 38.13 ± 0.19, 37.63 ± 0.18, 37.37 ± 0.17 and 37.28 ± 0.22 °C at 30, 60, 90 and 120 min, respectively.

Table 9. Effects of GH5763 and aspirin on the licking time of mice in the formalin test: early phase

Group	Dose (mg/kg)	Licking time (s)	Inhibition of Licking response (%)
Control	-	91.56 ± 9.34	-
Aspirin	300	69.71 ± 6.39*	24
GH5763	10	61.42 ± 8.68**	33
	20	54.44 ± 4.27**	40
	40	41.66 ± 2.19***	54

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Control = received 5% Tween 80 only.

Table 10. Effects of GH5763 and aspirin on the licking time of mice in the formalin test: late phase

Group	Dose (mg/kg)	Licking time (s)	Inhibition of Licking response (%)
Control	-	97.76 ± 11.24	-
Aspirin	300	42.13 ± 7.21***	57
GH5763	10	68.32 ± 9.35*	30
	20	44.85 ± 10.51**	54
	40	26.68 ± 8.41***	73

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Control = received 5% Tween 80 only.

Table 11. Effects of GH5763 and aspirin on yeast- induced hyperthermia in rats

Group	Dose (mg/kg)	Rectal temperature (°C)					
		Before Yeast injection	18 h after yeast injection				
			Time after medication (min)				
			0	30	60	90	120
Control	-	37.38 ± 0.24	39.28 ± 0.19	39.18 ± 0.18	39.02 ± 0.26	39.08 ± 0.16	39.17 ± 0.16
Aspirin	300	37.43 ± 0.14	39.37 ± 0.31	38.47 ± 0.28*	38.07 ± 0.14**	37.73 ± 0.14***	37.47 ± 0.27***
GH5763	10	37.62 ± 0.16	39.03 ± 0.07	38.13 ± 0.19**	37.63 ± 0.18***	37.37 ± 0.17***	37.28 ± 0.22***

Test drugs were orally administered 18 h after yeast injection.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from the rectal temperature 18 h after yeast injection : * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Control = received 5% Tween 80 only.

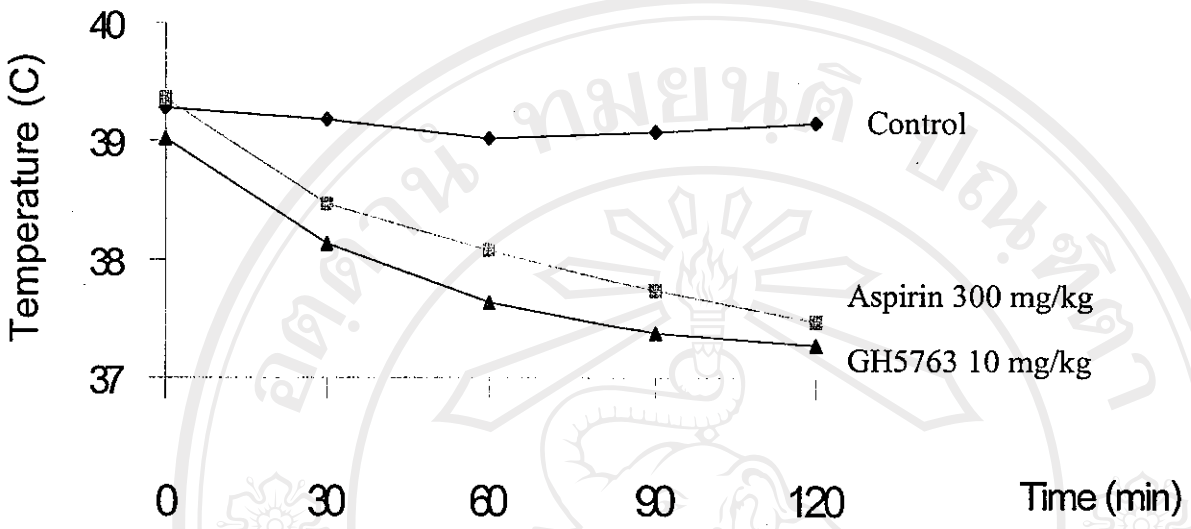

Figure 12 Effects of GH5763 and aspirin on yeast-induced hyperthermia in rats